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GM/CA-CAT Canted Undulator Beamlines for Macromolecular Crystallography: Current and Future Capabilities

Bob Fischetti
Associate Director, GM/CA-CAT
Biosciences Division
Argonne National Laboratory

January 24, 2007

APS Cross Cut Review of Biological Sciences

Funding and Community Served

GM/CA CAT is funded by the US National Institutes of Health's partnering institutes the National Institute of General Medical Sciences (GM), and the National Cancer Institute (CA) to build and operate a facility for macromolecular crystallography.

Beamtime is allocated in the following categories

- GM special grantees – Protein Structure Initiative (20.5%)
- CA special grantees – Structure based drug design (12.5%)
- General user facility (including DOE 25%) (42.0%)
- CAT Director, SAB members, R&D and staff research (25.0%)

GM/CA CAT Staff

Administration

Janet Smith
Director

Robert Fischetti
Associate Director

Sheila Trznadel
Admin Specialist

Crystallographic Support

Ward Smith

Nukri Sanishvili

Michael Becker

Naga Venugopalan

Computing Support

Sergey Stepanov

Oleg Makarov

Mark Hilgart

Engineering & Technical Support

Shenglan Xu

Derek Yoder

Rich Benn

Steve Corcoran

Scientific & Technical Vision

Wide energy tunability for MAD

3.5 - 35 keV (wavelength 3.5 - 0.35 Å)

Variable beam size to match to sample size

Easily varied in the range 10 - 200 μm

Small beam capability

Down to 10 μm routinely

High stability of beam position and wavelength

Small beam stable throughout a user's experiment

Wavelength tunable without beam position changing

User-friendly beamline operation

Easily understood & operated computer interface

Automation to speed sample screening

For both difficult and high-throughput projects, and for beamline setup

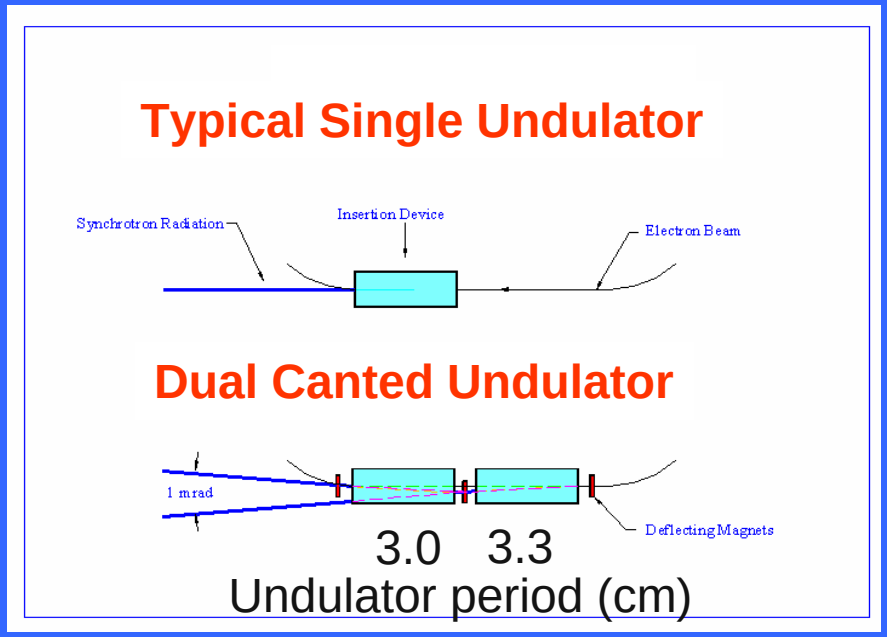
Not just high throughput

Larger macromolecules & complexes, small crystals, weakly diffracting crystals such as membrane proteins

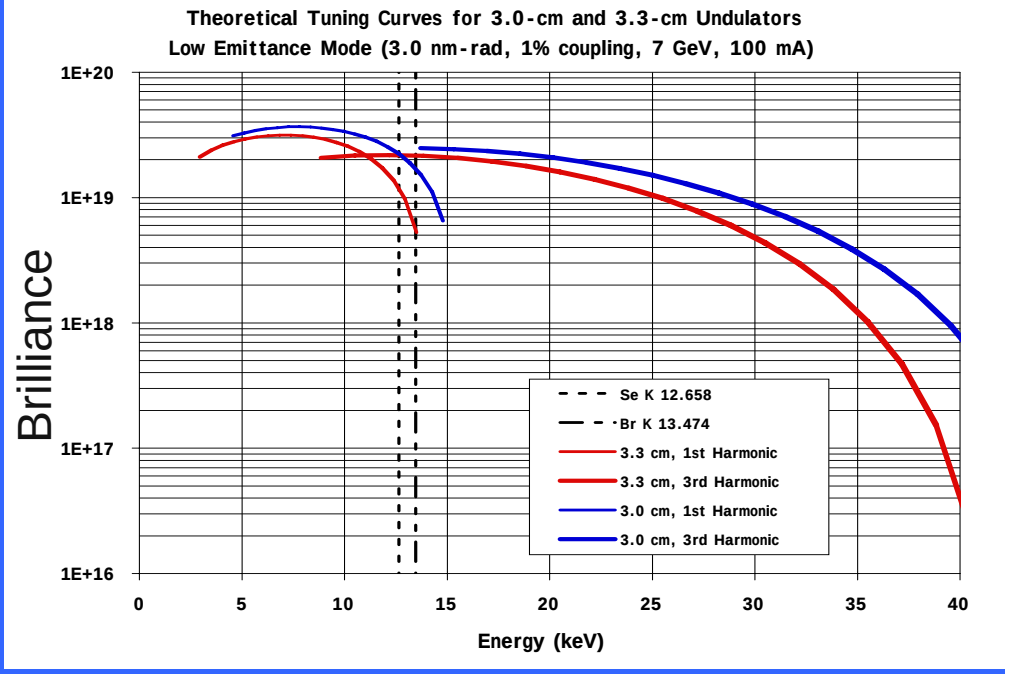
GM/CA-CAT Capabilities

- Two independent, rapidly tunable, canted-undulator beamlines
  - 23-ID-B: 3.3 cm undulator, energy range: 3.5 – 20 keV
  - 23-ID-D: 3.0 cm undulator (optimized for Se and Br), energy range: 5.0 – 35 keV with monochromator crystal change
- One rapidly tunable bending magnet beamline, energy range: 3.5 – 35 keV with monochromator crystal change (being commissioned)
- Small, stable beams on ID-lines - bimorph mirrors arranged in a K-B geometry
  - Small focal size 20 x 70 microns
  - Homogeneous beam profile at focus and at sample
- ALS-style sample automounters on all 3 beamlines
- Excellent sample viewing through an on-axis microscope
- Convenient user interface: SSRL's Blu-Ice GUI adapted to EPICS control environment
- Mini-beam apparatus for small (< 10 micron) crystals
- CCD detectors: MAR 300 on ID-line, MAR 225 on BM-line

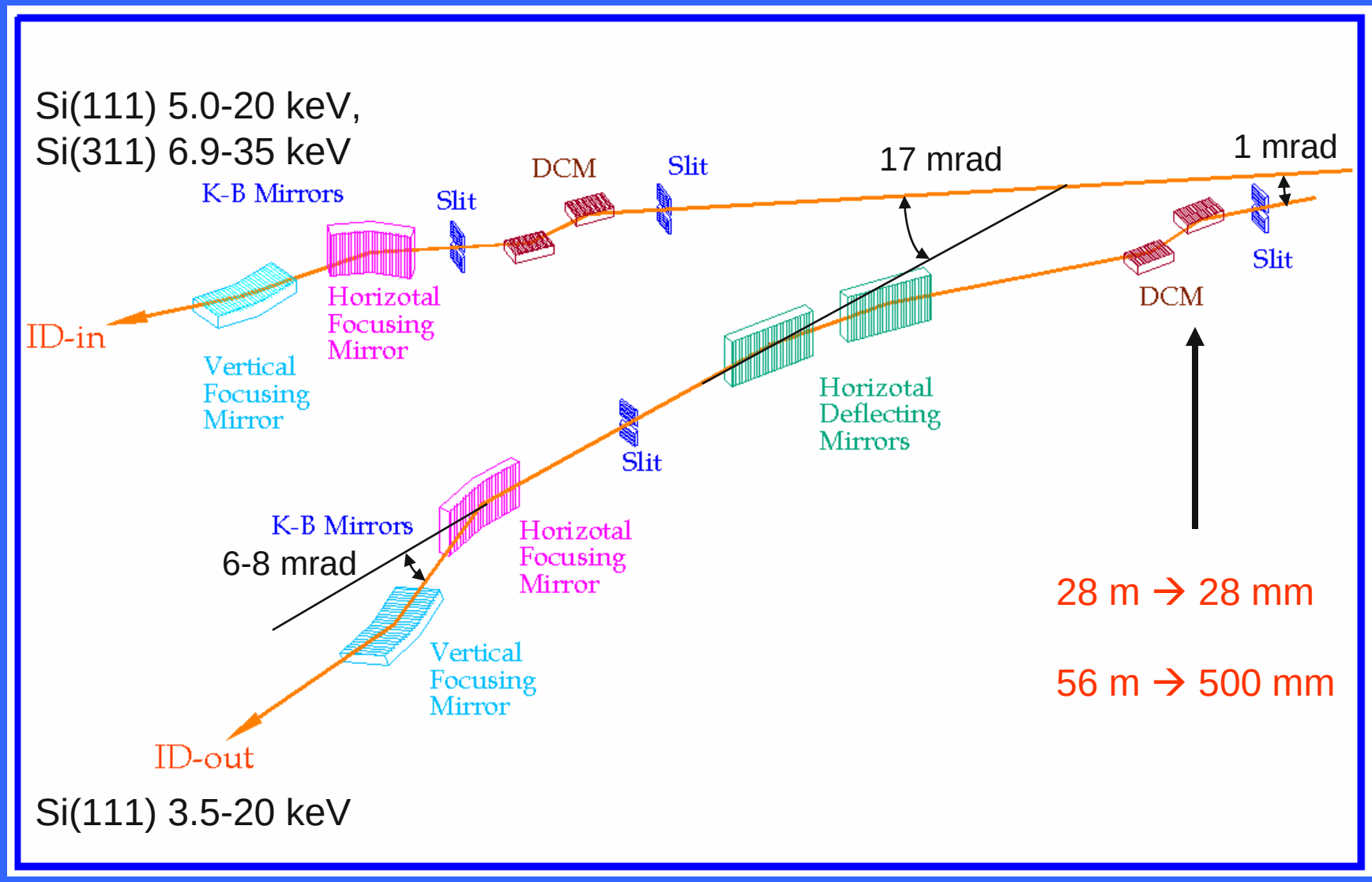
1st APS Sector Based on Hard X-ray Dual Canted Undulators



Independent steering: 3 RF-BPMs
Stable: 1 X-ray BPM at 22 m
Increased flexibility:
- 3.3 cm – standard UA
- 3.0 cm – optimized for Se and Br



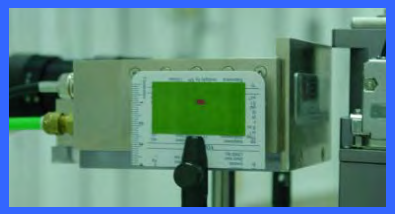
Schematic of Canted ID Beamlines



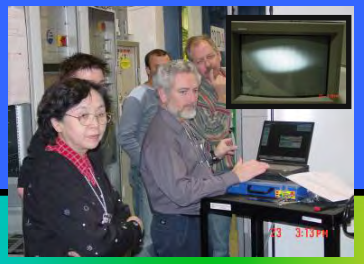
Milestones

ACCEL construction contract signed	May 20, 2003
White light delivered to FOE	August 11, 2003
White beam to ID-C	April 20, 2004
First monochromatic light ID_{in}	June 14, 2004
First diffraction data on ID_{in}	***July 8, 2004***
First user on ID_{in}	July 12, 2004
KB “bimorph” mirrors installed ID_{in}	August 16, 2004
First monochromatic light from dual canted undulators	***February 16, 2005***
First diffraction experiments on ID_{out}	December 21, 2005
First monochromatic light on BM (under going commissioning)	February 23, 2006
Sample automounter made available to users	***December 1, 2006***
First mini-beam data set collected on users sample	***December 16, 2006***

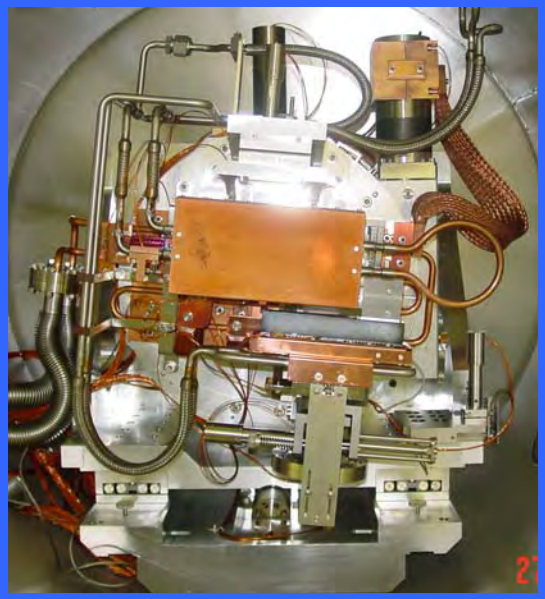







ACCEL - Double Crystal Monochromator



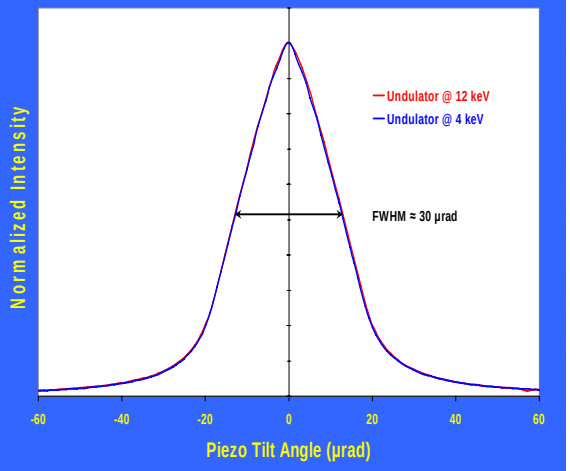
- Constant exit height
- Long 2nd crystal to minimize tune error
- 2nd crystal translates in Bragg perpendicular direction
- 1st and 2nd crystals are both indirectly, cryogenically cooled
- Compton scatter shield around 1st crystal and 2nd crystal mount for improved thermal stability
- In-vacuum Huber 430 rotation axis – avoids rotary feedthrough
- Si (111) or Si(311) for higher energy and/or resolution

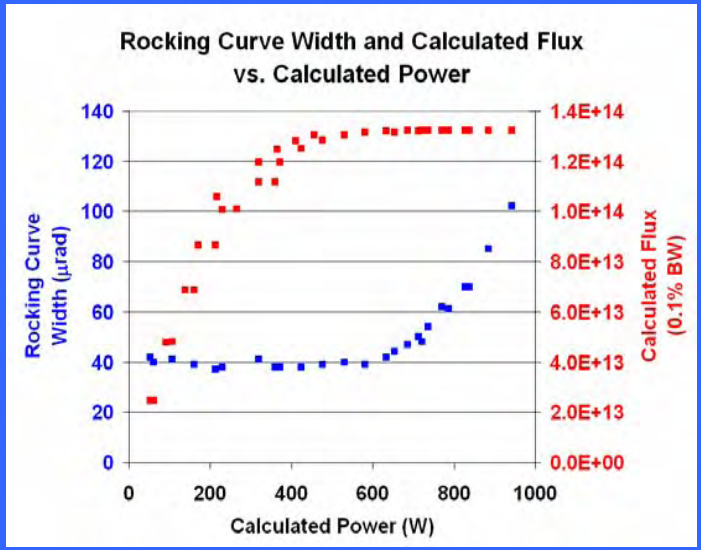
Thin web under cut

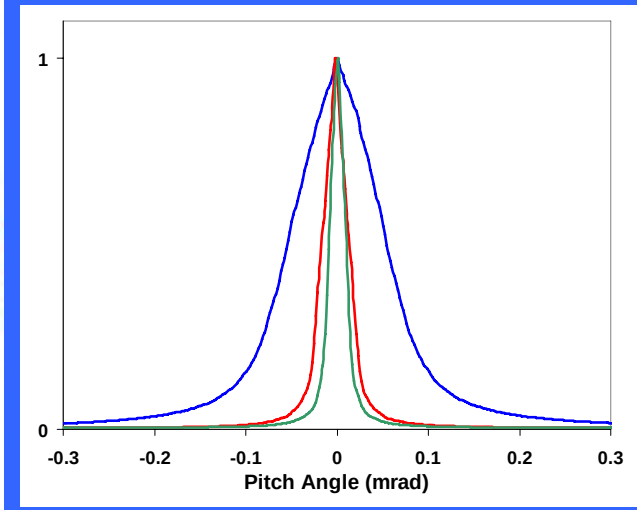
Reduces absorbed power and amount of Compton scattering produced

Monochromator Performance

- Energy resolution in good agreement with Si (111)
- High heat load tolerance
  - No broadening of rocking curve with 600 W on 1st crystal at 100 mA
  - Can accept 90% of central cone at 200 mA (FEA calculations)
- Minimal rocking curve dispersion or “detuning” of 1st and 2nd crystals *vs.* energy
- Calibration procedures for non-Bragg axes to stabilize beam position to less than +/- 5 µm over the range 4 – 20 keV
- Spectroscopy-grade edge scans
- BPM after DCM for intensity feedback and YAG crystal for visualizing the beam







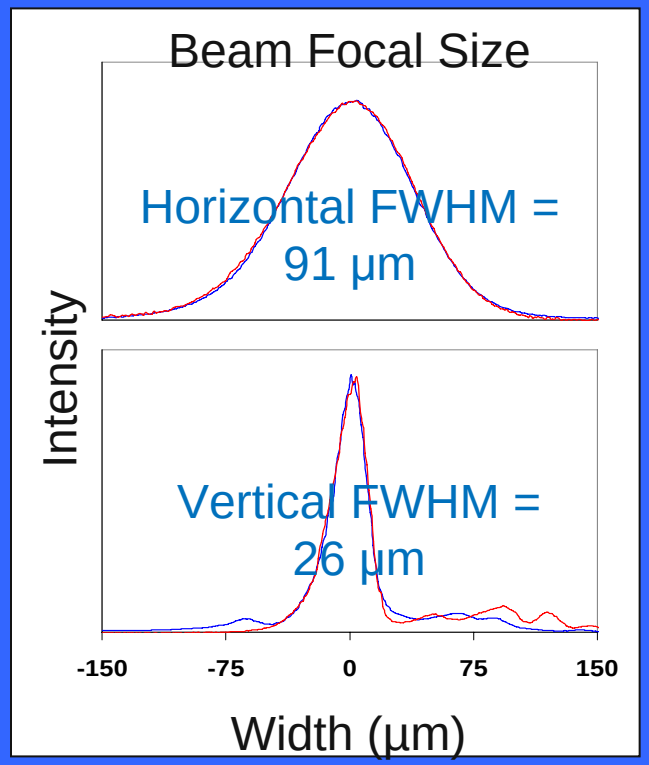
K-B "Bimorph" Mirrors Performance – achromatic focusing

Early results – August 2004

Focused at the sample
21 x 62 microns (FWHM)

Focused 1 m downstream of sample
26 x 91 microns (FWHM)

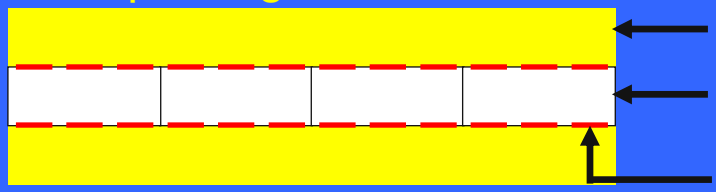
Energy Independence
Beam profile at 12 kev (red)
Beam profile at 18 keV (blue)

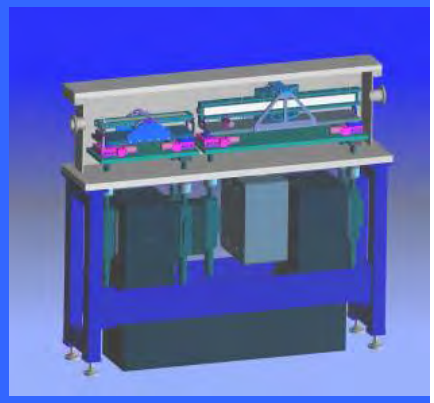


Uniform electrode voltages – set curvature

Differential electrode voltages – correct slope error

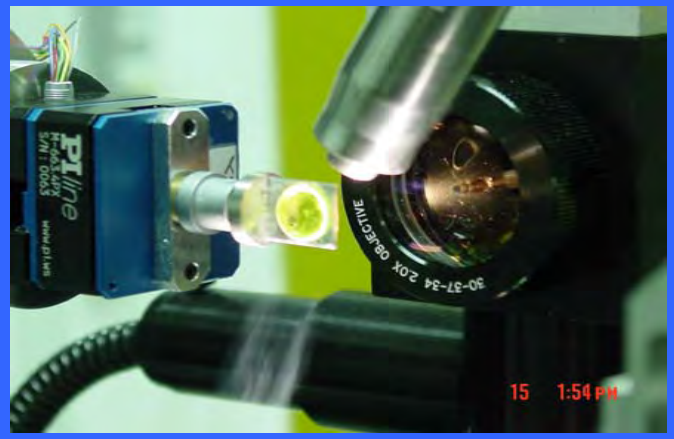
Bimorph longitudinal cross section

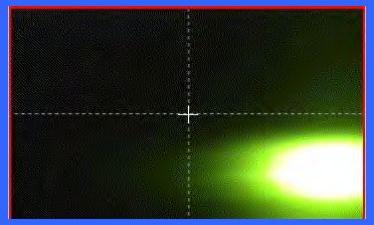


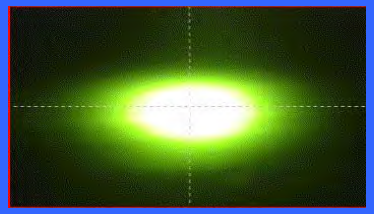


On-Axis Crystal Viewing Optics – YAG to visualize the beam

- Motor controlled 16X zoom
- Beam coincident with optical axis (through high numerical aperture objective lens)
- Field of view - 2.4 mm X 3.2 mm down to 0.15 mm X 0.20 mm
- Front and backlight







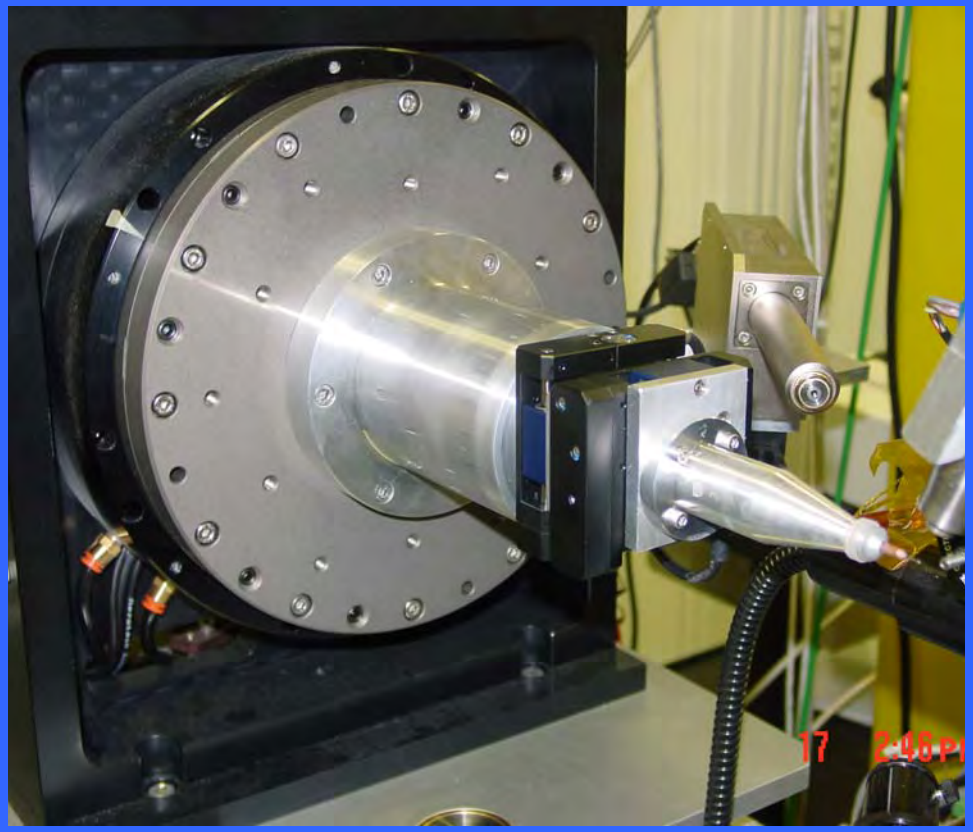
150 µm

200 µm

Easy alignment of sample to beam

- Center rotation axis on cross hair
- Move beam to cross hair with slide bar control of mirror pitch angle (piezo actuators)
- Mount sample and center on cross hair

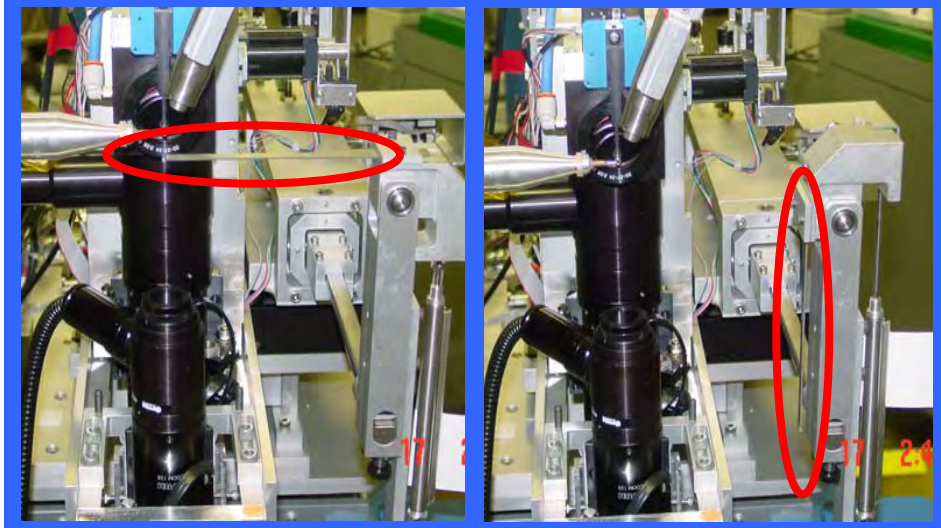
Precision Omega Axis – small sphere-of-confusion



- AeroTech ABR1000 air bearing:
  <1 um wobble
- XY sample positioner:
  2 – 3 micron sphere of confusion

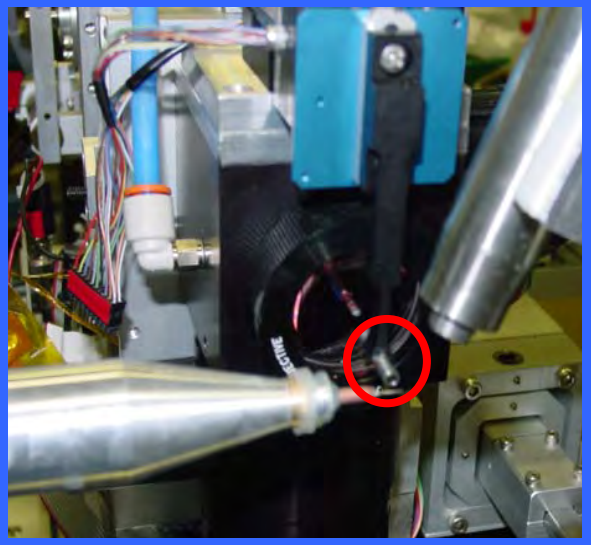
Sample Environment

Beamstop “Flipper” Assembly



Beam stops automatically moves out of the way when hutch door opens, and repositions when the door closes.

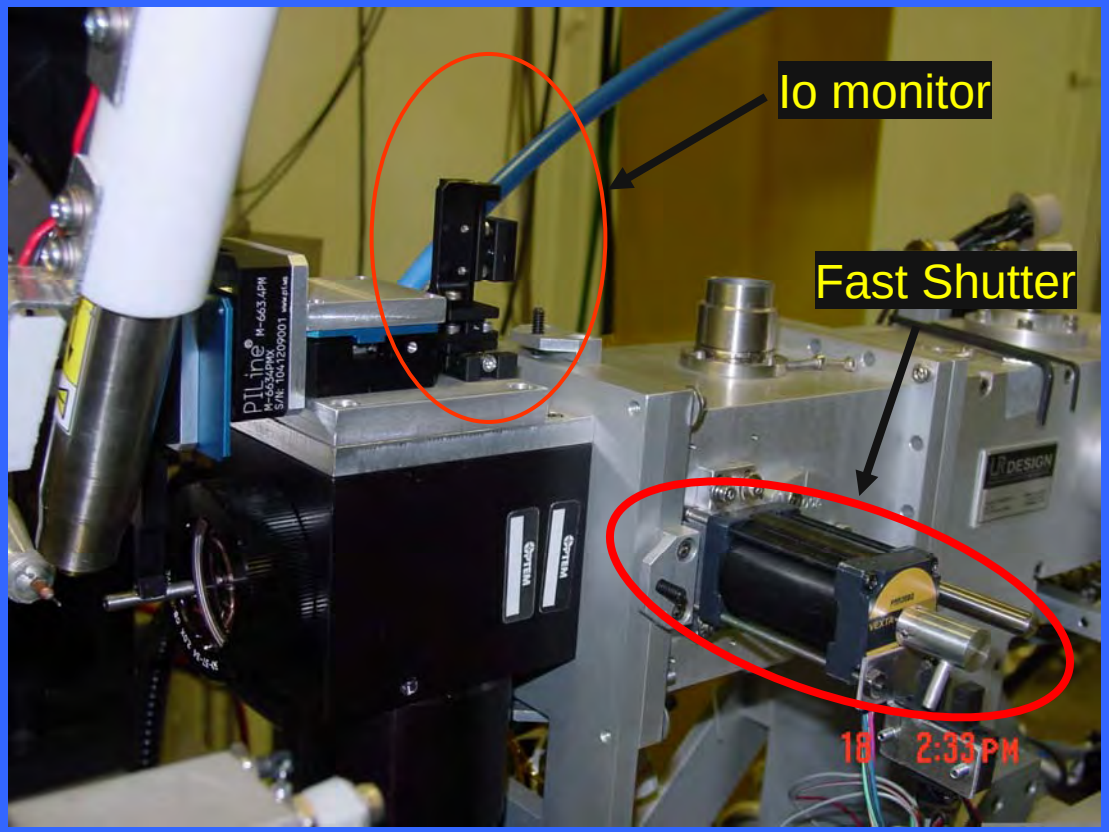
Motorized scatter guard

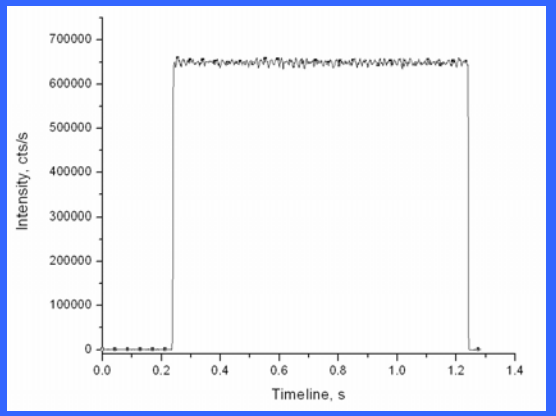


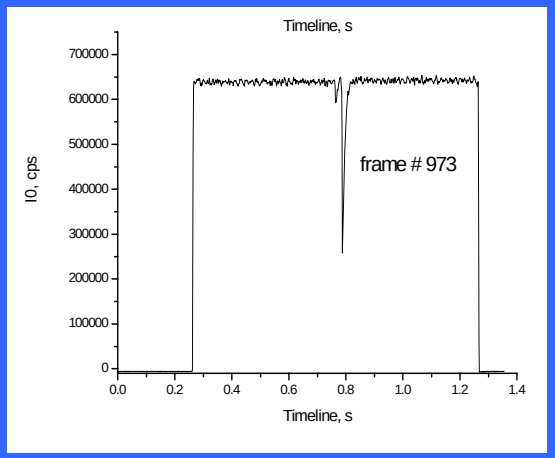
Scatter guard moves out of optical path to allow visualization of the crystal. Data collection software repositions before taking data.

I0 monitor after fast shutter

- Al scatter foil and PIN Diode
- Positioned after fast shutter
- Provides beam intensity measurement during exposure







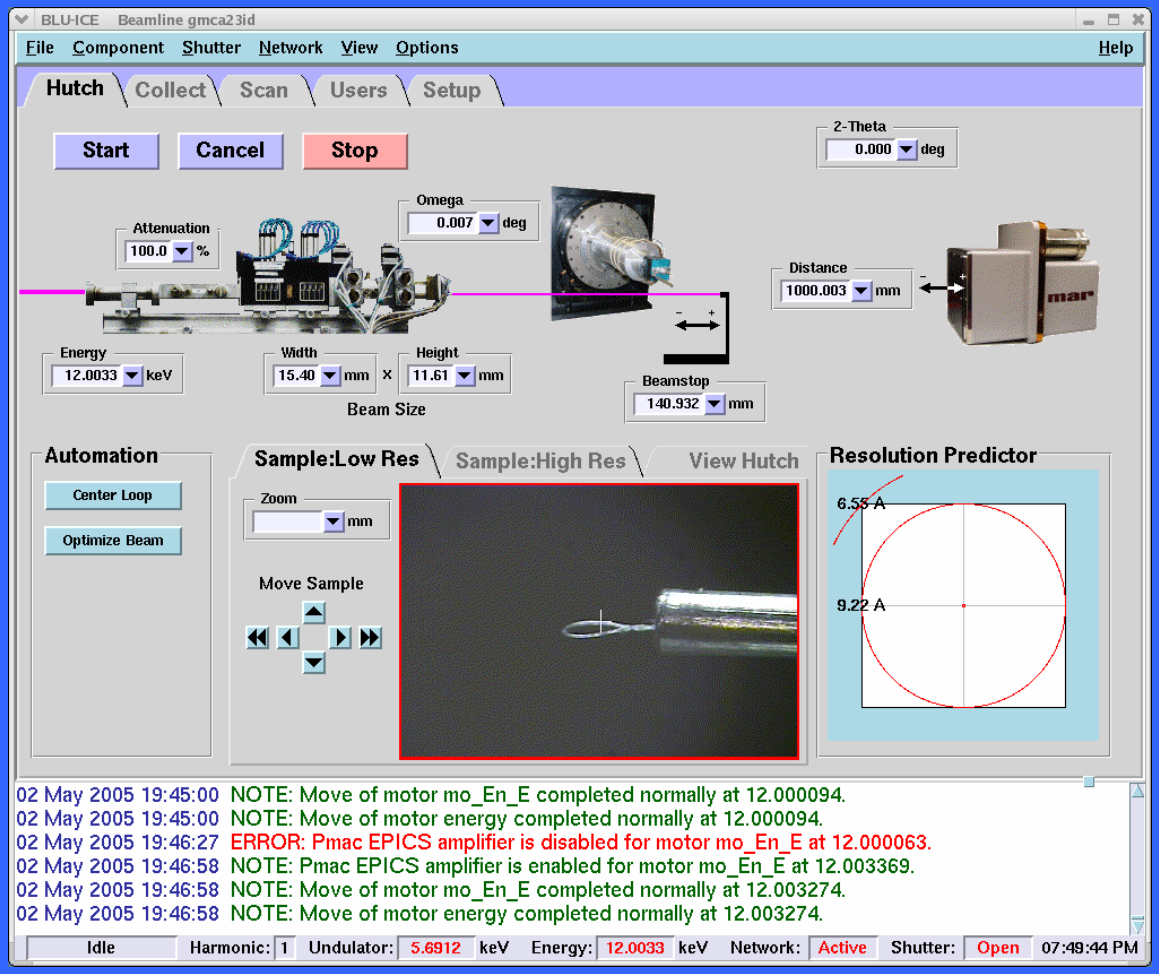
Captured Top-Up

Automated sample handling

Modified ALS robot design, collaborated with LRDesign to incorporate improvements suggest by Thomas Earnest, Carl Cork and others, and to customize for our goniometry.

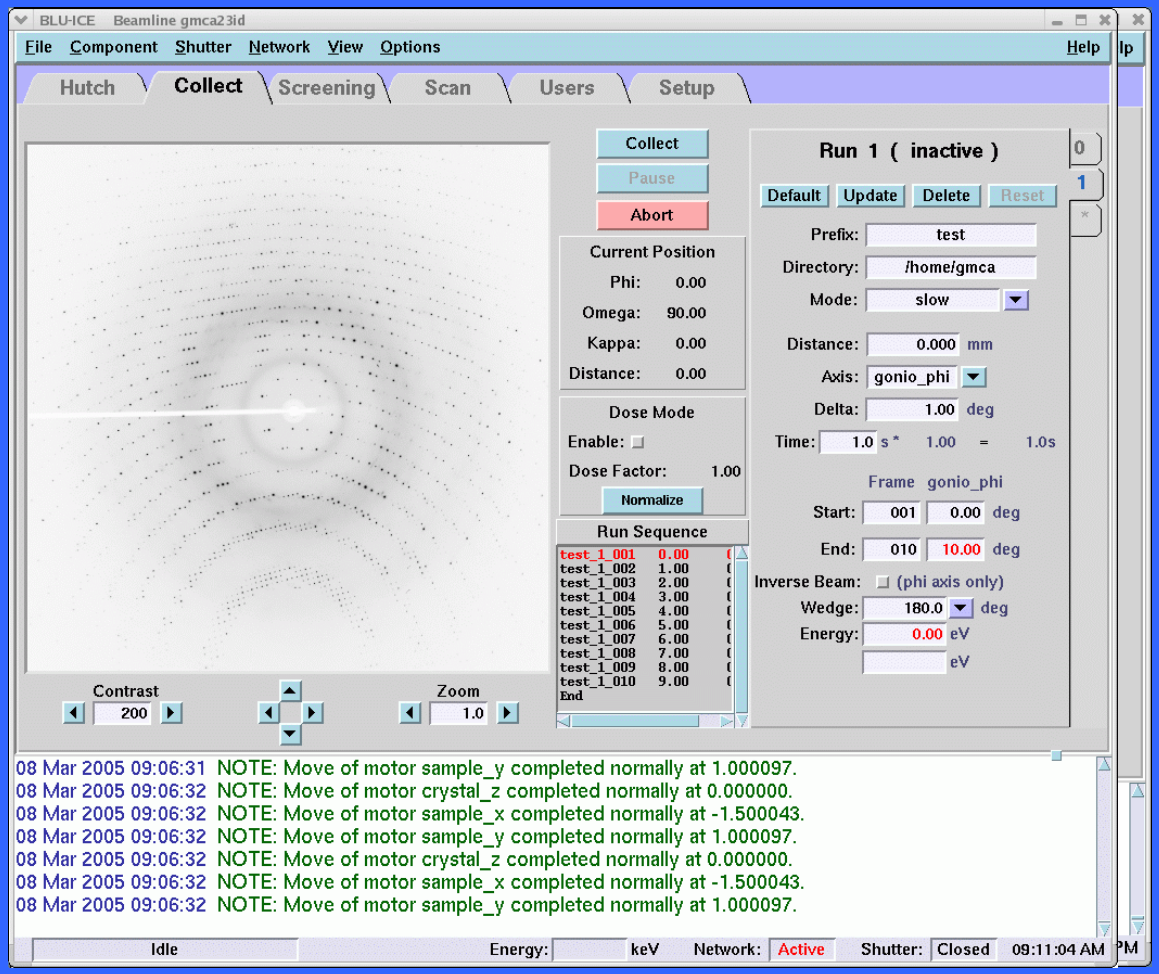
- Installed on all three beamlines
- Available to users on ID-lines
- Compatible with either ALS/SSRL (96 pins), or Rigaku (72 pins) pucks
- Screening takes < 3 min/crystal with “point & click” centering
- Implementing automated sample centering (hiring Sadhir Babu Pothineni from Victor Lamzin’s group (EMBL/Hamburg) - XREC
- Configuration allows manual mounting

Blu-Ice user interface



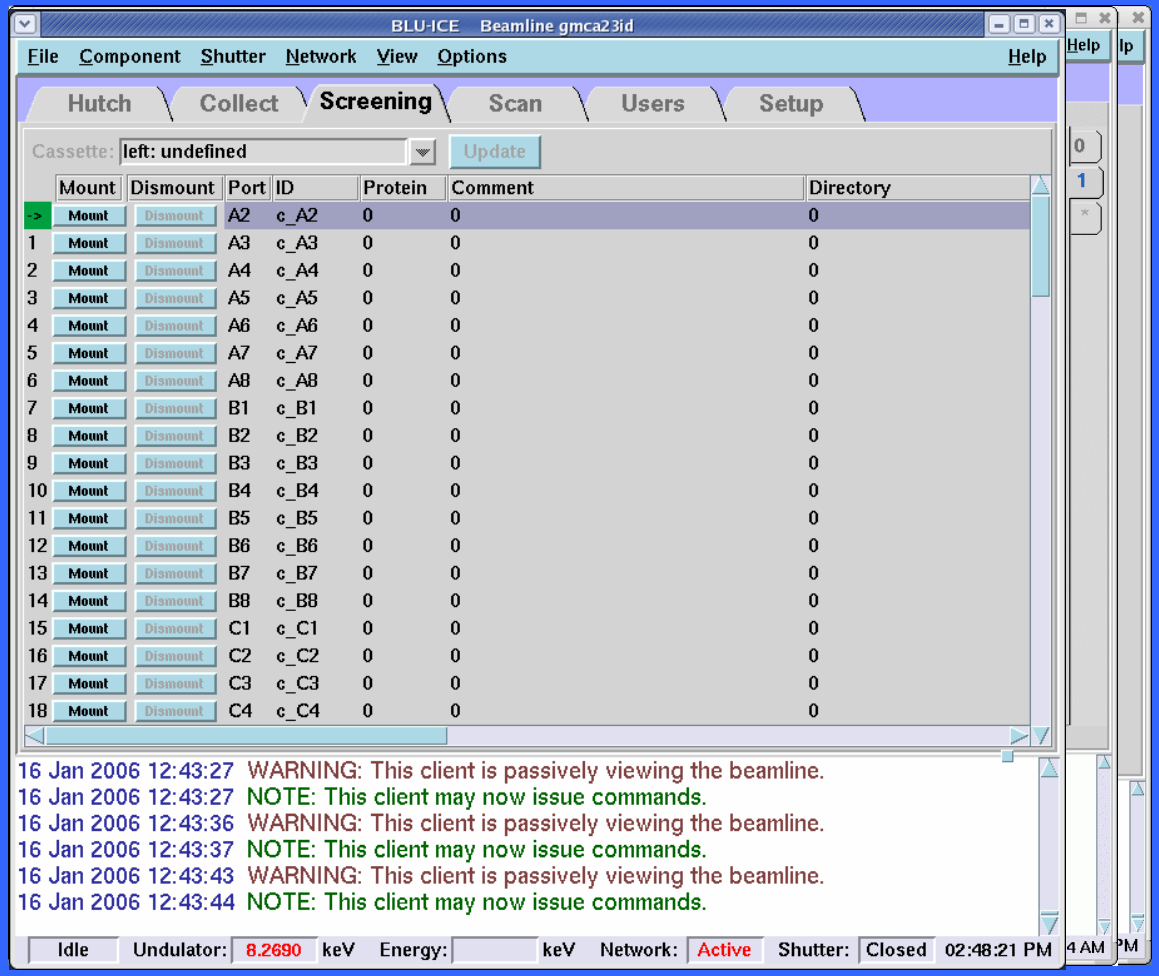
SSRL's Blu-Ice GUI adapted to EPICS control environment

Blu-Ice user interface



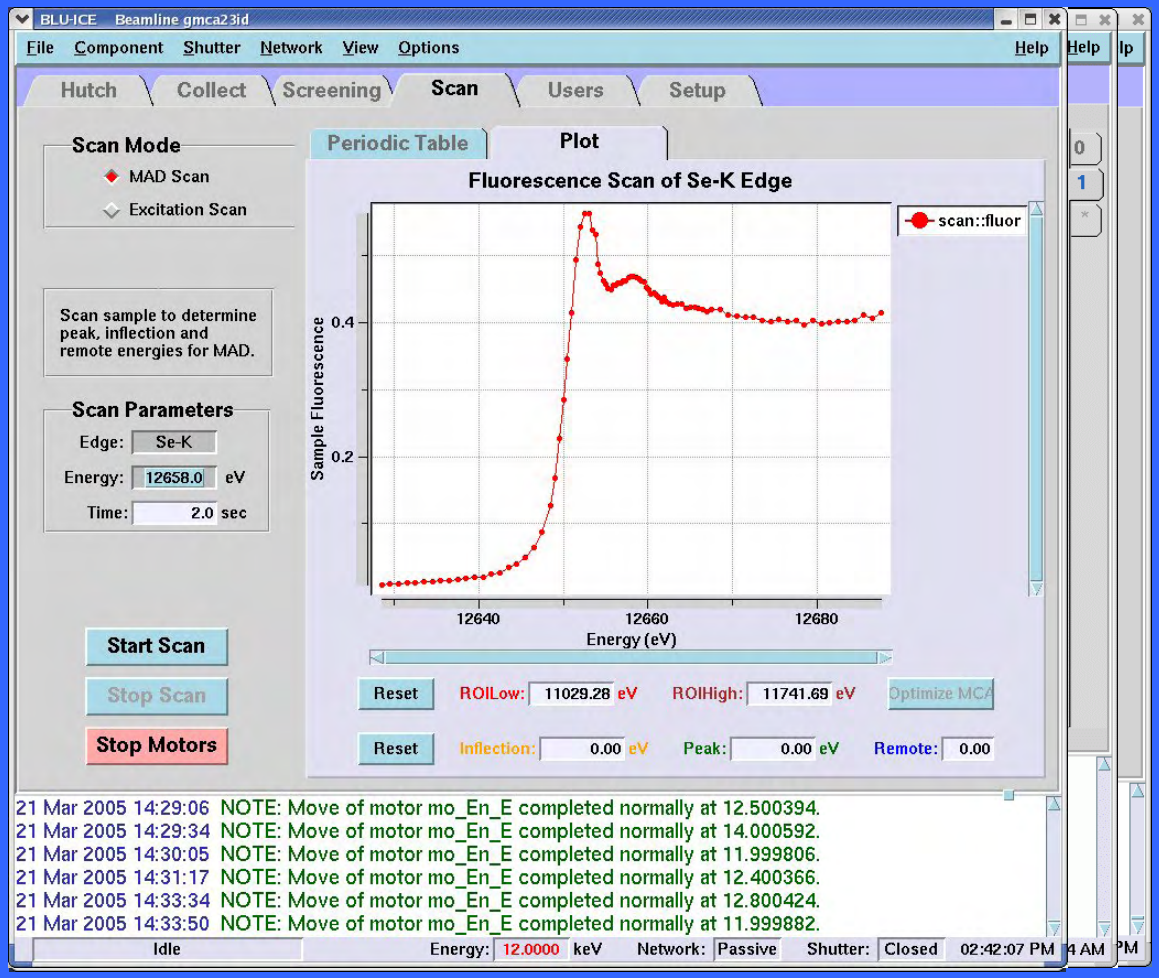
SSRL's Blu-Ice GUI adapted to EPICS control environment

Blu-Ice user interface



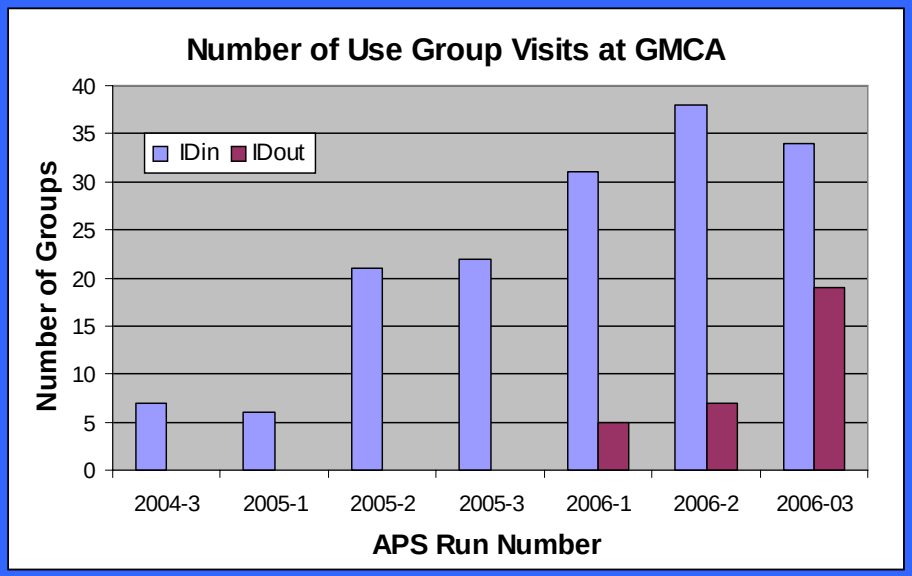
SSRL's Blu-Ice GUI adapted to EPICS control environment

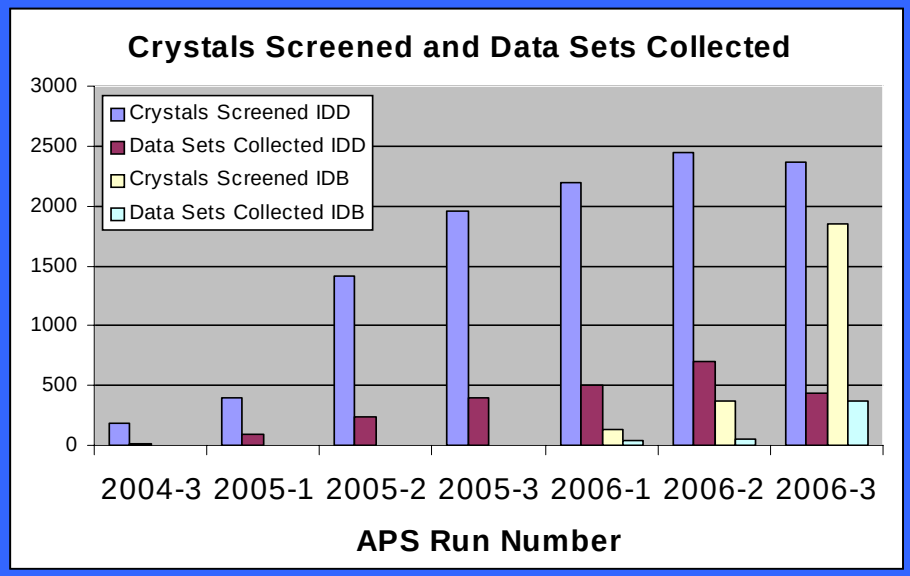
Blu-Ice user interface



SSRL's Blu-Ice GUI adapted to EPICS control environment

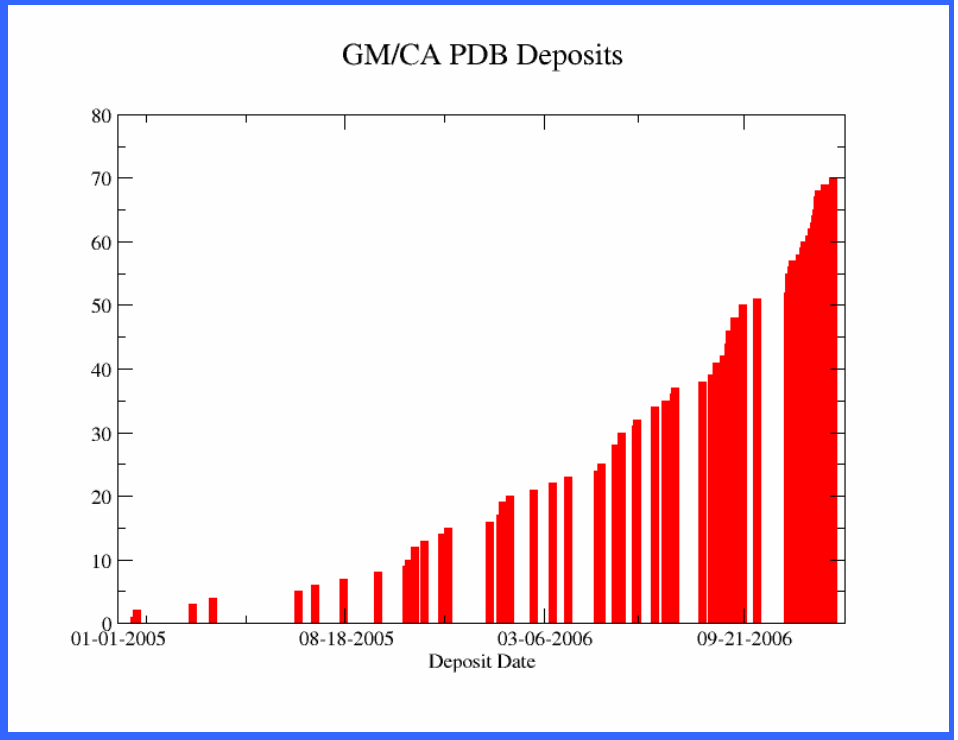
Ramp Up of the User Program





Total Number for all Runs

	Crystal Screened	Sets Collected	PDBs
ID-D	10979	2382	62
ID-B	2355	469	8
Total	13334	2852	70

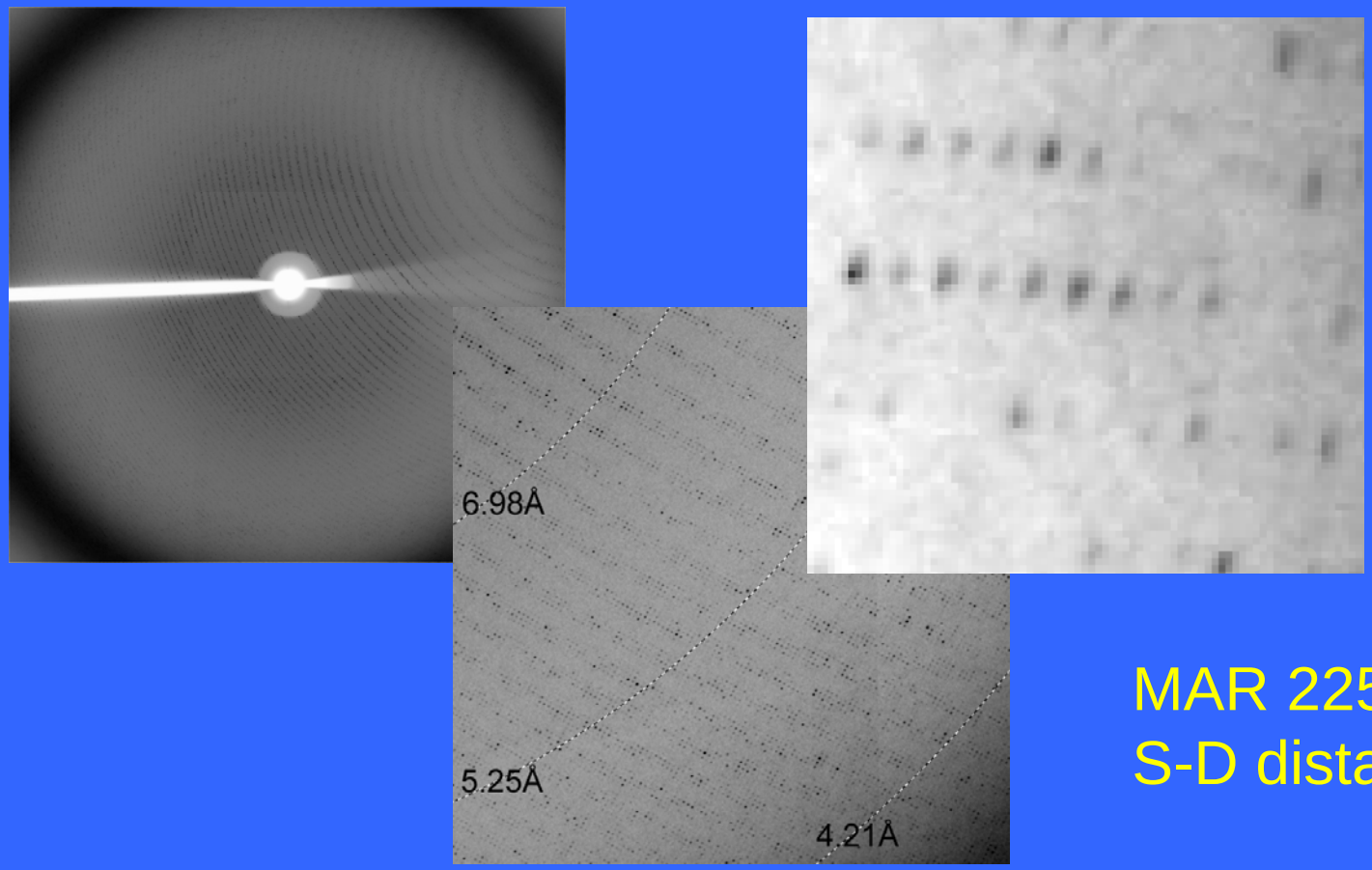


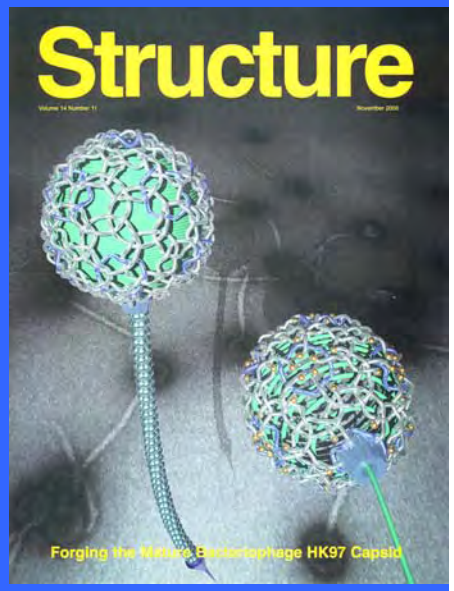
GM/CA-CAT Accomplishments

- 26 Articles in refereed journals
  - *1-Cell*
  - *1-Nature*
  - *7-PNAS*
  - *2-Nature Chem Biol,*
  - *1-Nature Struc Mol Biol*
- 1 Cover (Structure)
- 70 Structures deposited (PDB)
- 44 Invited talks
- 2 Awards
- 3 Conference proceedings
- 3 Press releases on the GM/CA facility (C&E News, R&D Magazine, NIH News)

Large Unit Cells

Diffraction pattern from HK97 virus capsid.
Unit cell dimensions: 1010 x 1010 x 732 Å





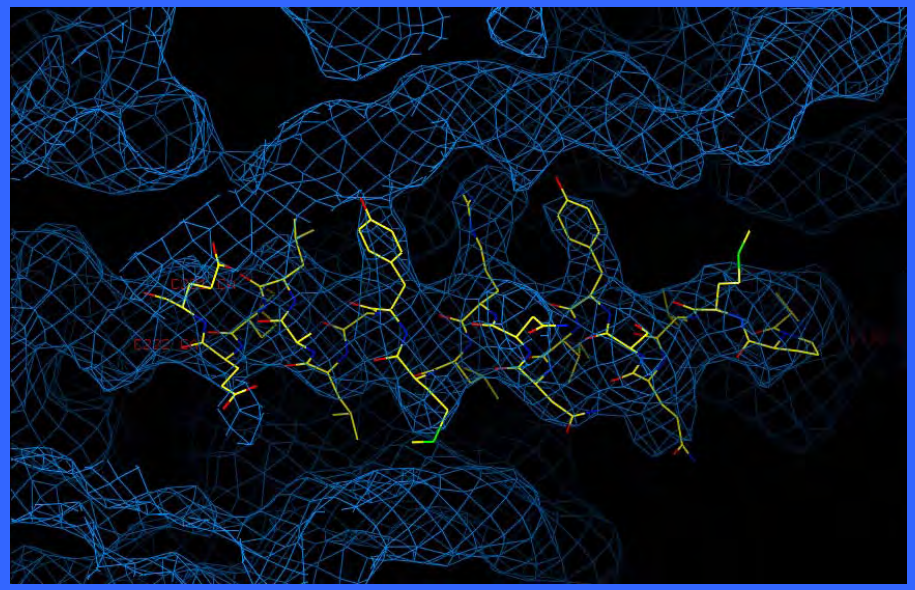
MAR 225
S-D distance 680 mm

L. Gan, *et al*. & J. E. Johnson *Structure* 14, 1655-65 (2006)

Large Unit Cells, continued

Model fitting into electron density map of HK97 capsid (Jack Johnson/Lu Gan)

"…Figure below shows a section of electron density from the 4.2Å map of Head II. The quality is superb and bulky side chains can be visualized even at this resolution…."



Mini-beam Capability

Requirements

- ~5 μm beam
- improved beam positional stability
- accurate beam position in visual field
- effective lighting for ~5 μm colorless samples
- sample position stable to < 5 μm
- goniometer center of rotation stable to < 5 μm
- easy alignment of sample to beam

All of the above attributes must be stable through sample changes.

Advantages of mini-beam

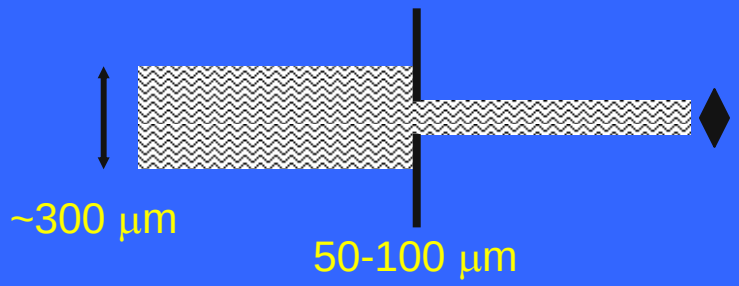
- Raster long crystals
- Select best part of crystal
- Reduced background
- Projects that only produce small crystals

Disadvantages of mini-beam

- Diffraction is weak form small crystals
- Radiation damage is still a factor

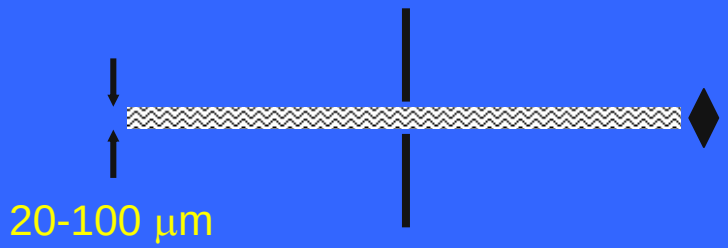
Smaller and smaller beams: the practical challenge

Most common approach to a small beam for crystallography: overfill an aperture



- Tolerates beam positional instabilities
- Misses lots of flux
- Increased background from slits
- Not suitable for micro-focus

Our approach to a small beam for crystallography: focus to desired size

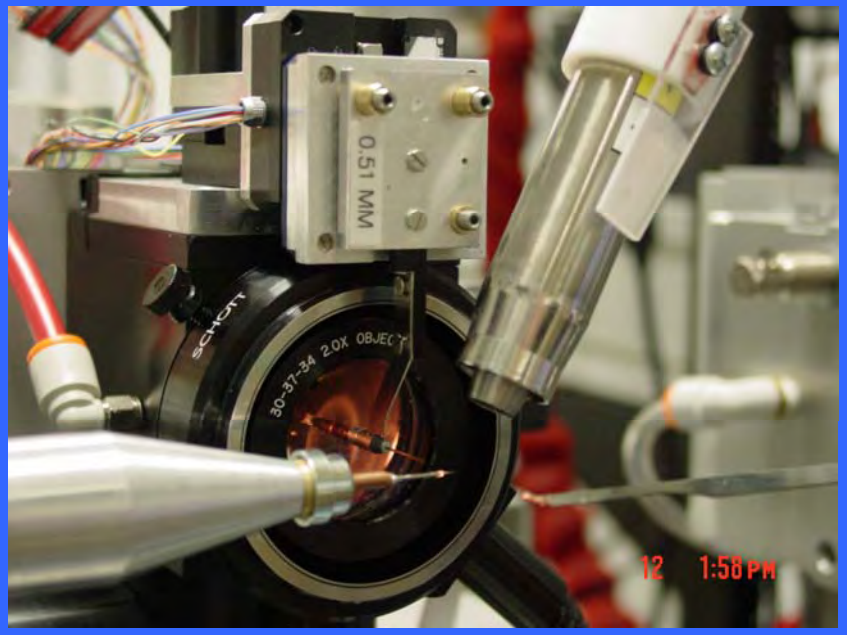


- Can provide high resolution data
- Captures lots of flux
- Good signal-to-noise
- Suitable for micro-focus
- Sensitive to beam positional instabilities

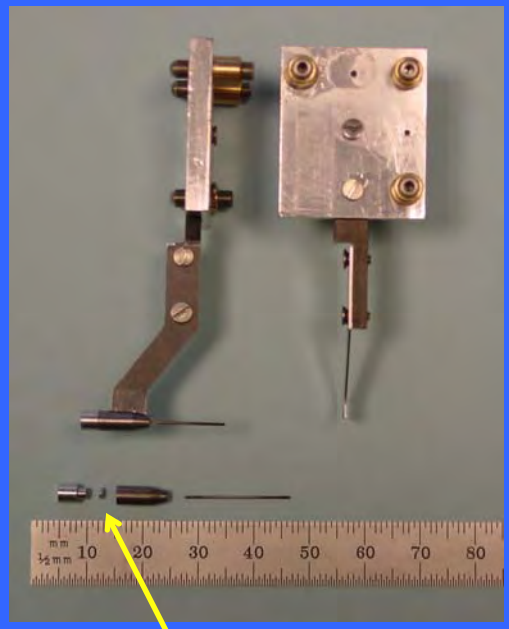
Mini-beam approach combines both: focus to small beam at the sample position and then aperture to the desired beam size (similar to ESRF ID13 and micro-diff)

- Beam defining slits are typically to far away (> 200 mm)
- Mostly reduce intensity but not size of beam at sample

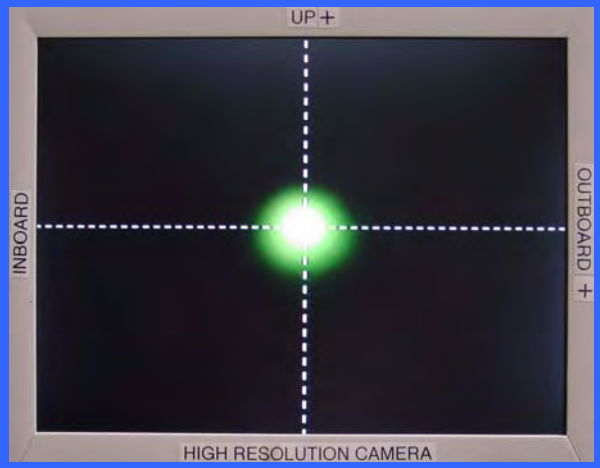
Mini-beam apparatus: small beam-defining pinholes



Kinematics mount
- rapidly interchangeability
- 15 minute alignment



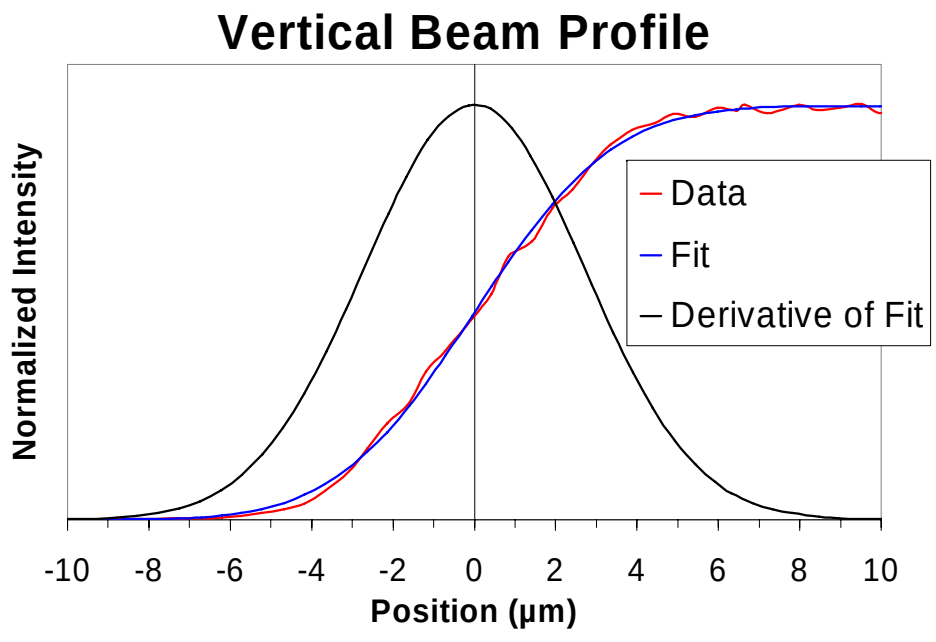
5, 10, 30 μm apertures



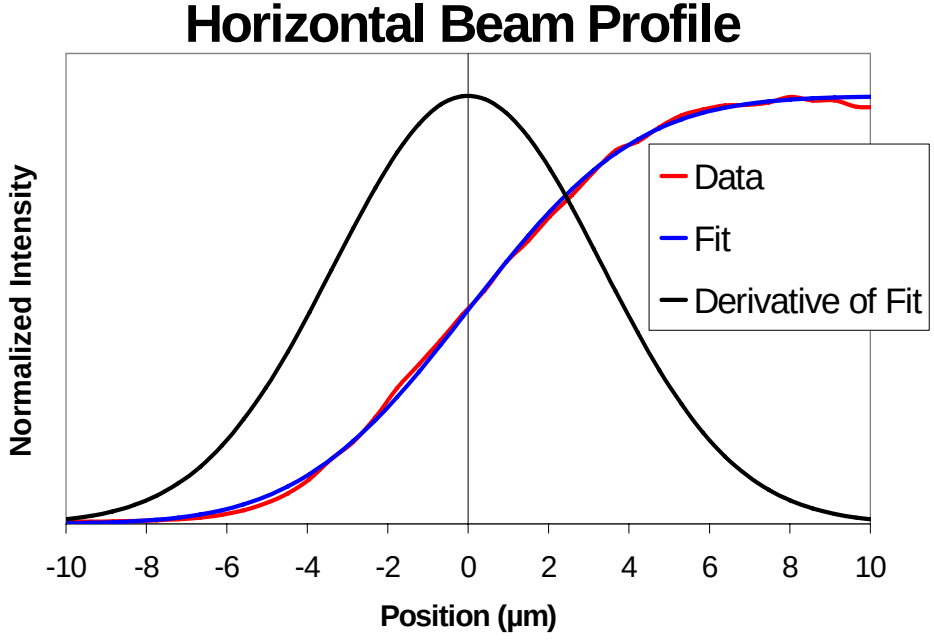
7 μm beam (FWHM)
Viewed on YAG crystal at sample position
Beam focused at sample position
10 μm aperture

Knife Edge Scans of "Mini-beam" – 10 micron pin hole

FWHM = 6.3 microns



FWHM = 7.8 microns



Flux through pin hole: ~ 1×10^{11} photons/sec/100 mA

Still need to attenuate!

5 micron pin hole – 3.8 x 6.8 microns FWHM (V x H)

Visualization of small crystals

7 x 7 x 15 μm^3 lysozyme crystal in 30 micron MiTeGen loop

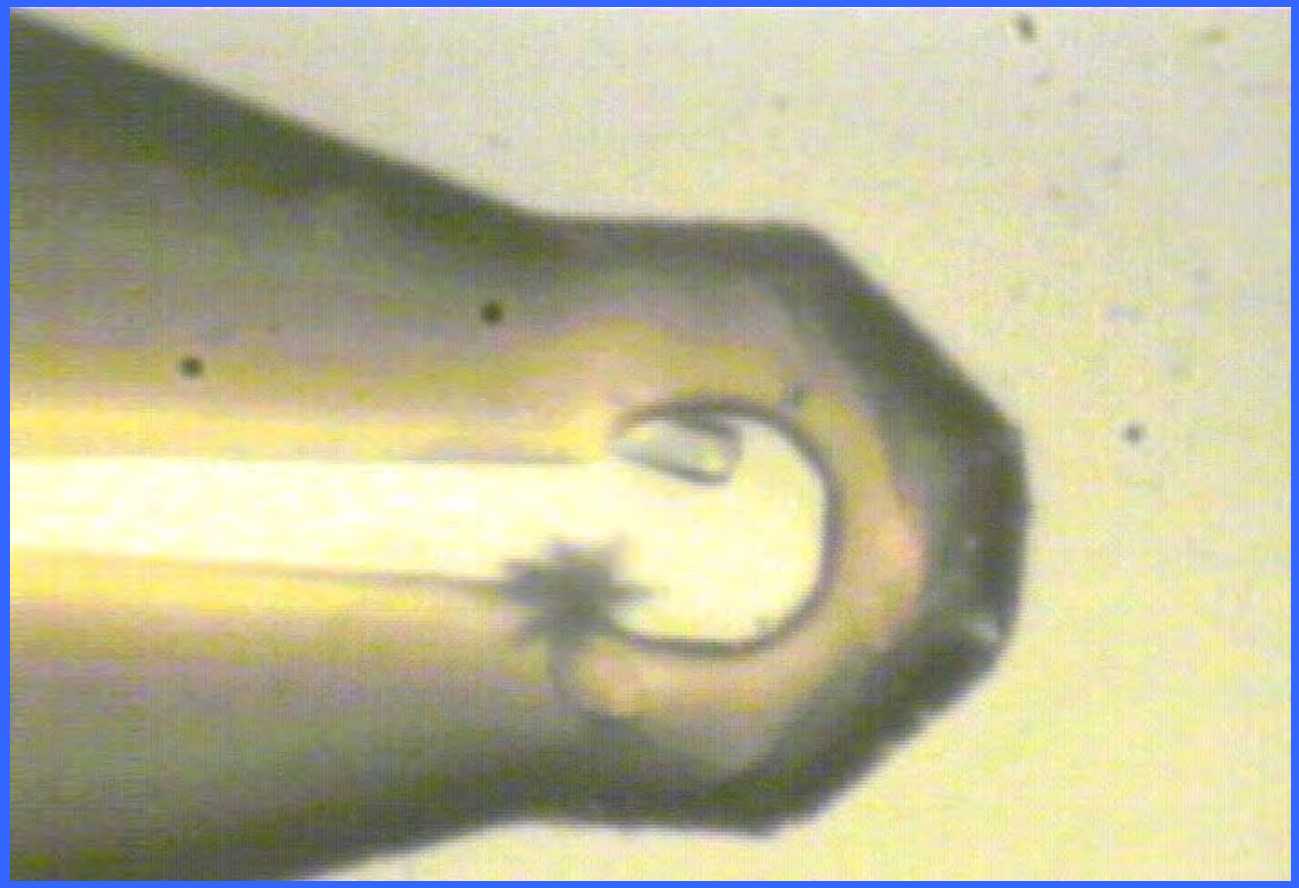
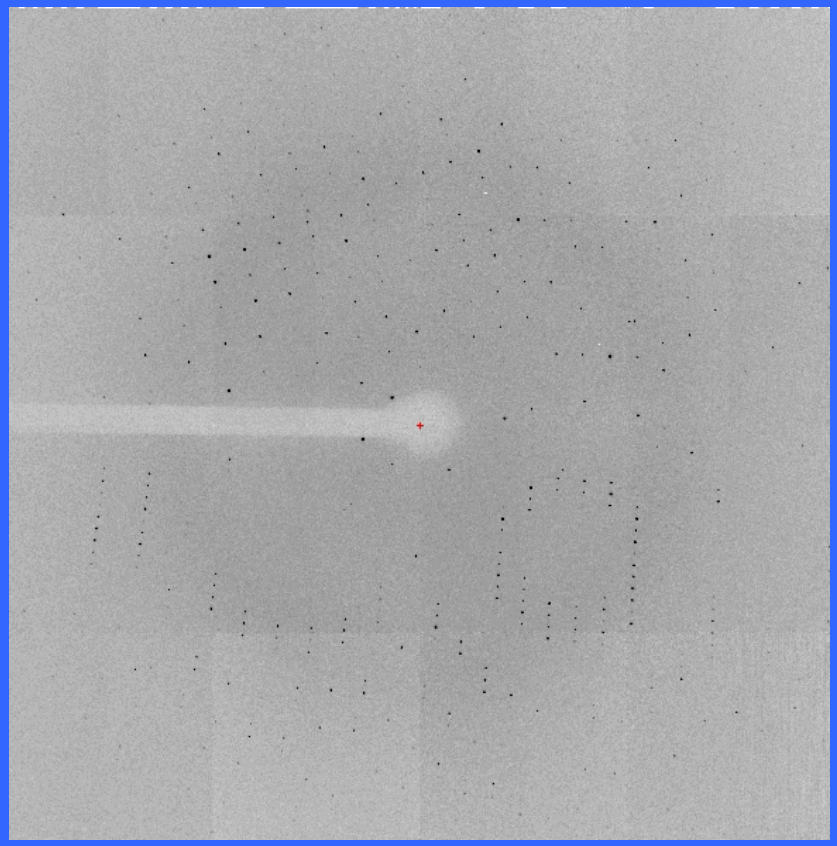


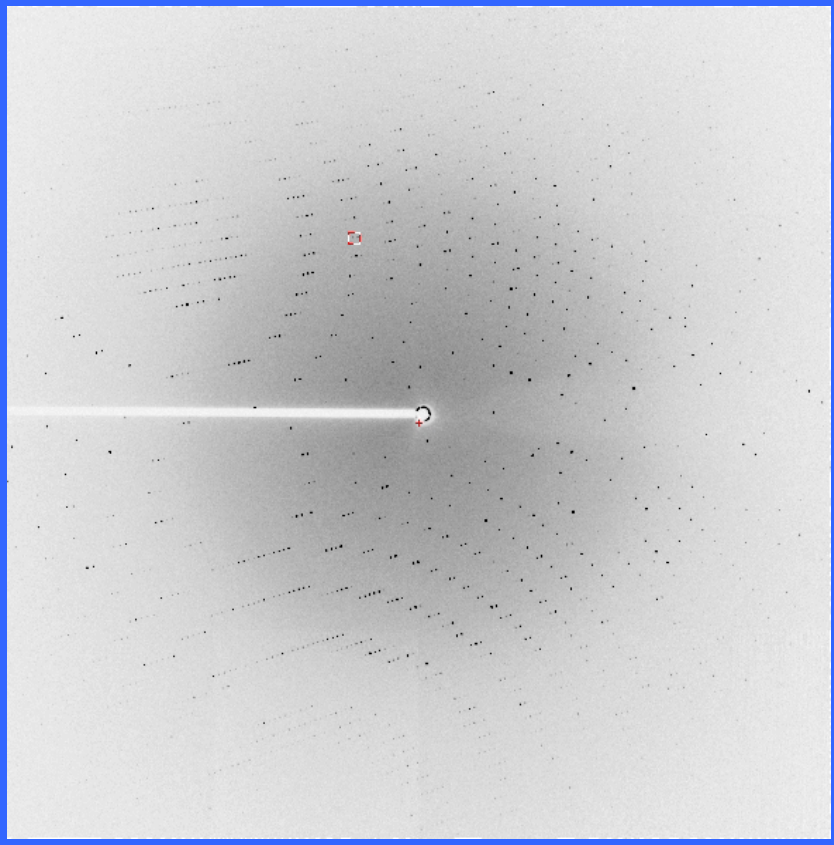
Image recorded with mini-beam apparatus in position

Diffraction patterns from small crystals

7x7x15 μm^3 lysozyme crystal



10x4x<4 μm^3 thaumatin crystal

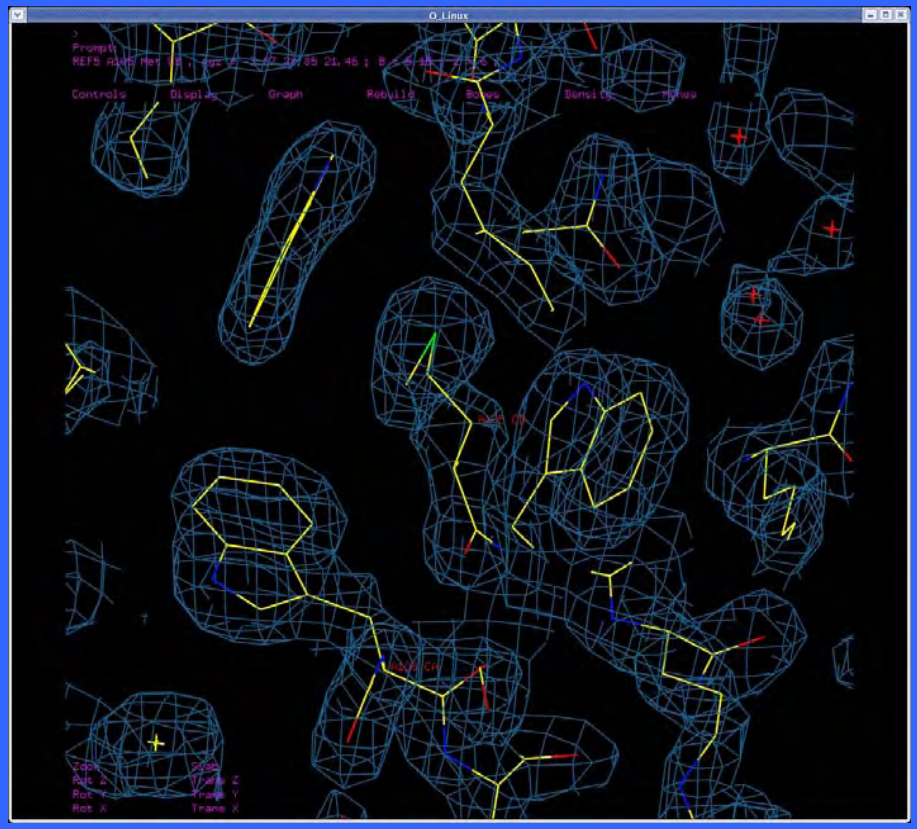


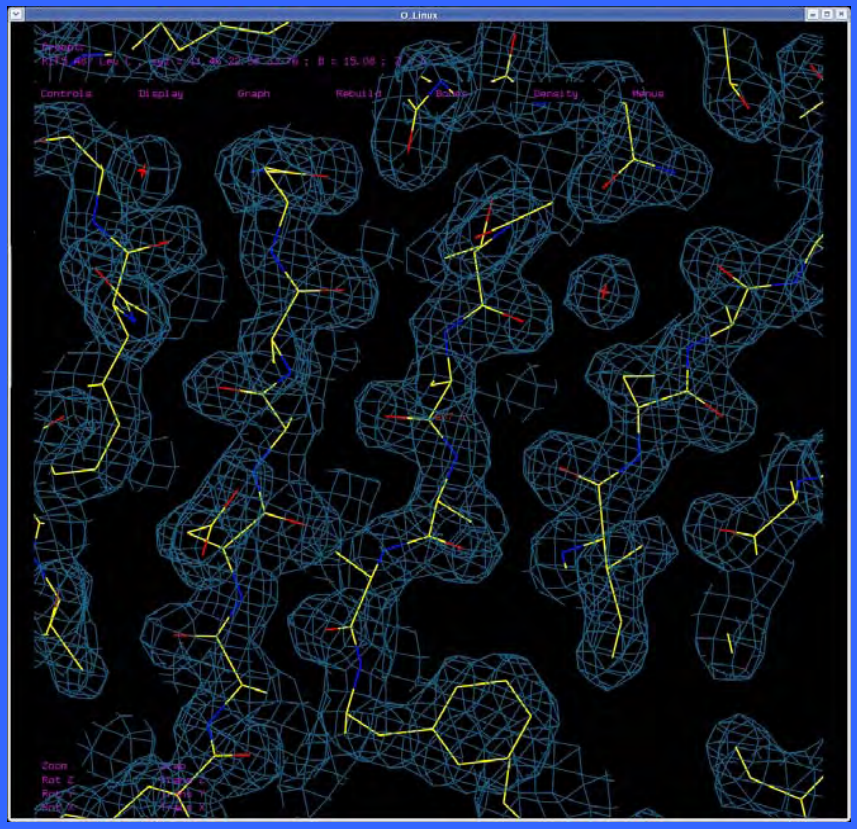
Diffraction from small crystals is weak, but measurable

Refined data from micro-crystals

Region of lysozyme structure refined at 2.25 Å resolution against the data collected from a single small crystal.

Region of thaumatin structure refined at 1.94 Å resolution against the data collected from 6 small crystals and then merged.

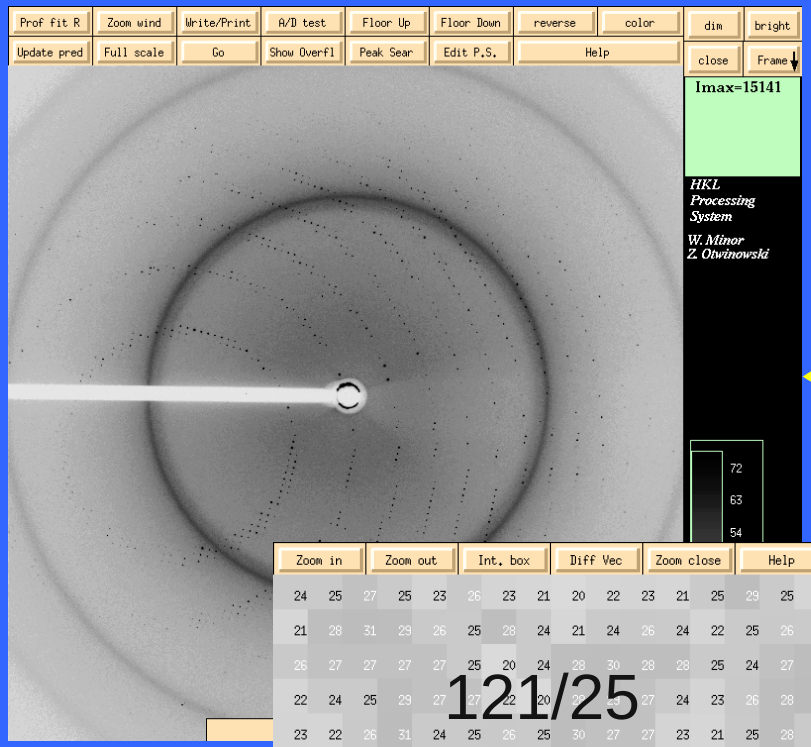


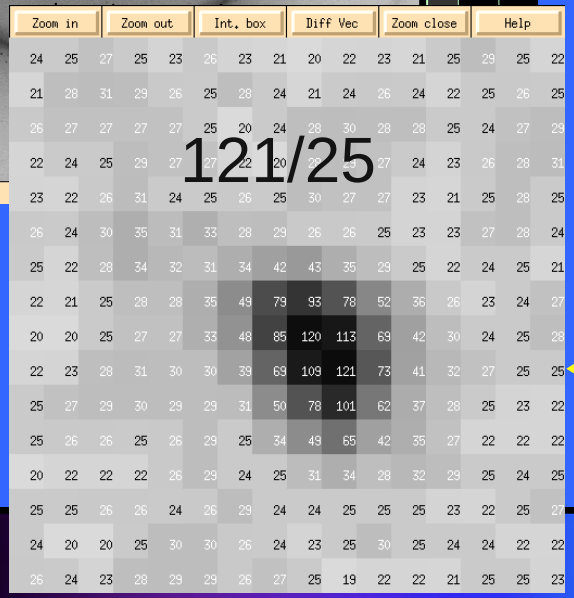


Electron densities are plotted with 1 sigma cut-off.

Improved Signal/Noise Ratio from Same Crystal

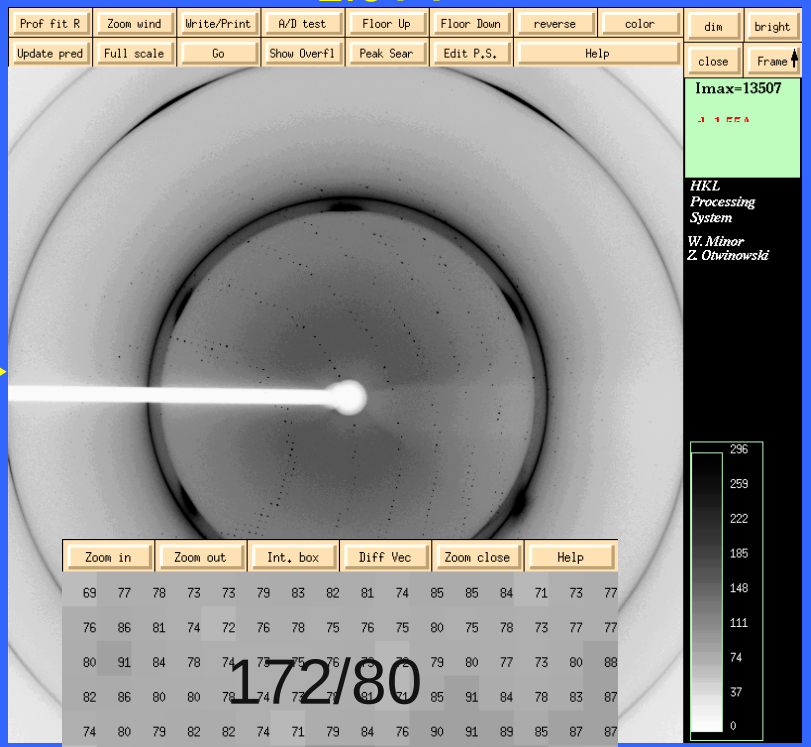
7-μm beam
2.4 Å

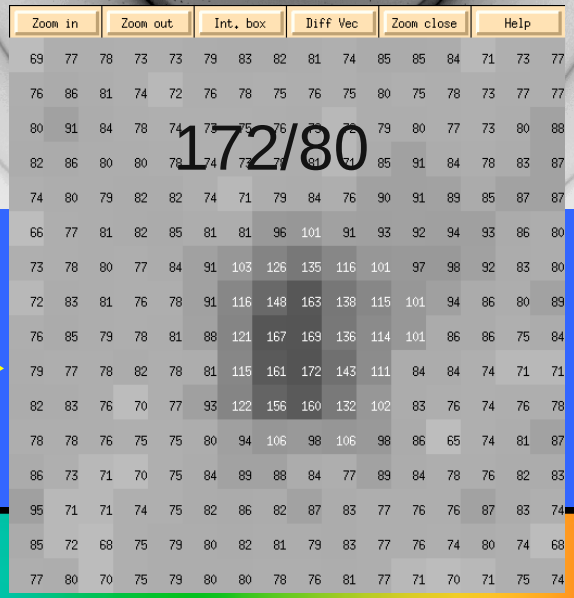




Same crystal & setting

75-μm beam
2.6 Å





Same reflection

Future

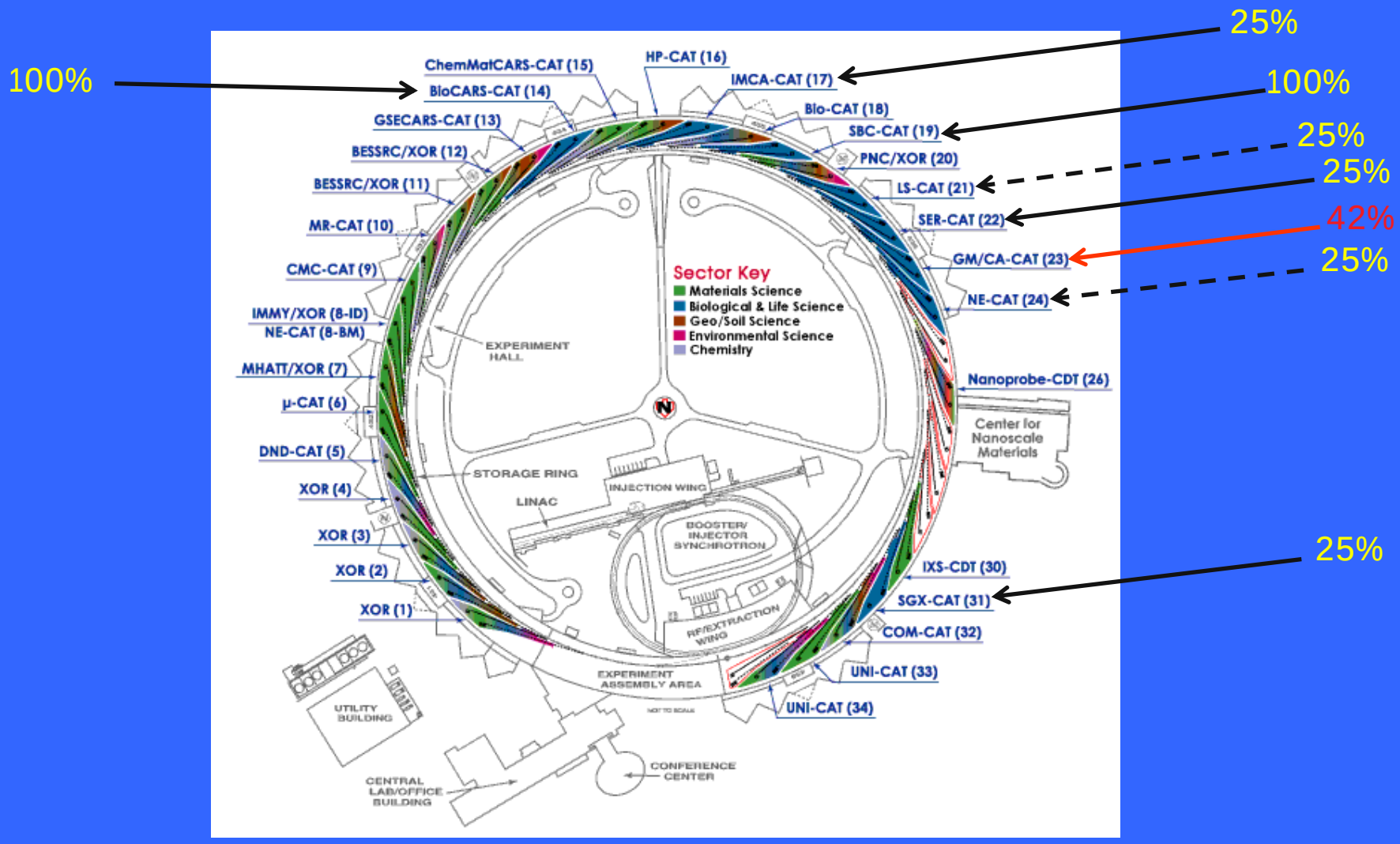
Improved efficiency

- Already have many automated tasks
- Developing automated alignment of optical components
- Developing automated focusing of mirrors
- Developing automated alignment of goniometry and centering of sample

Usage

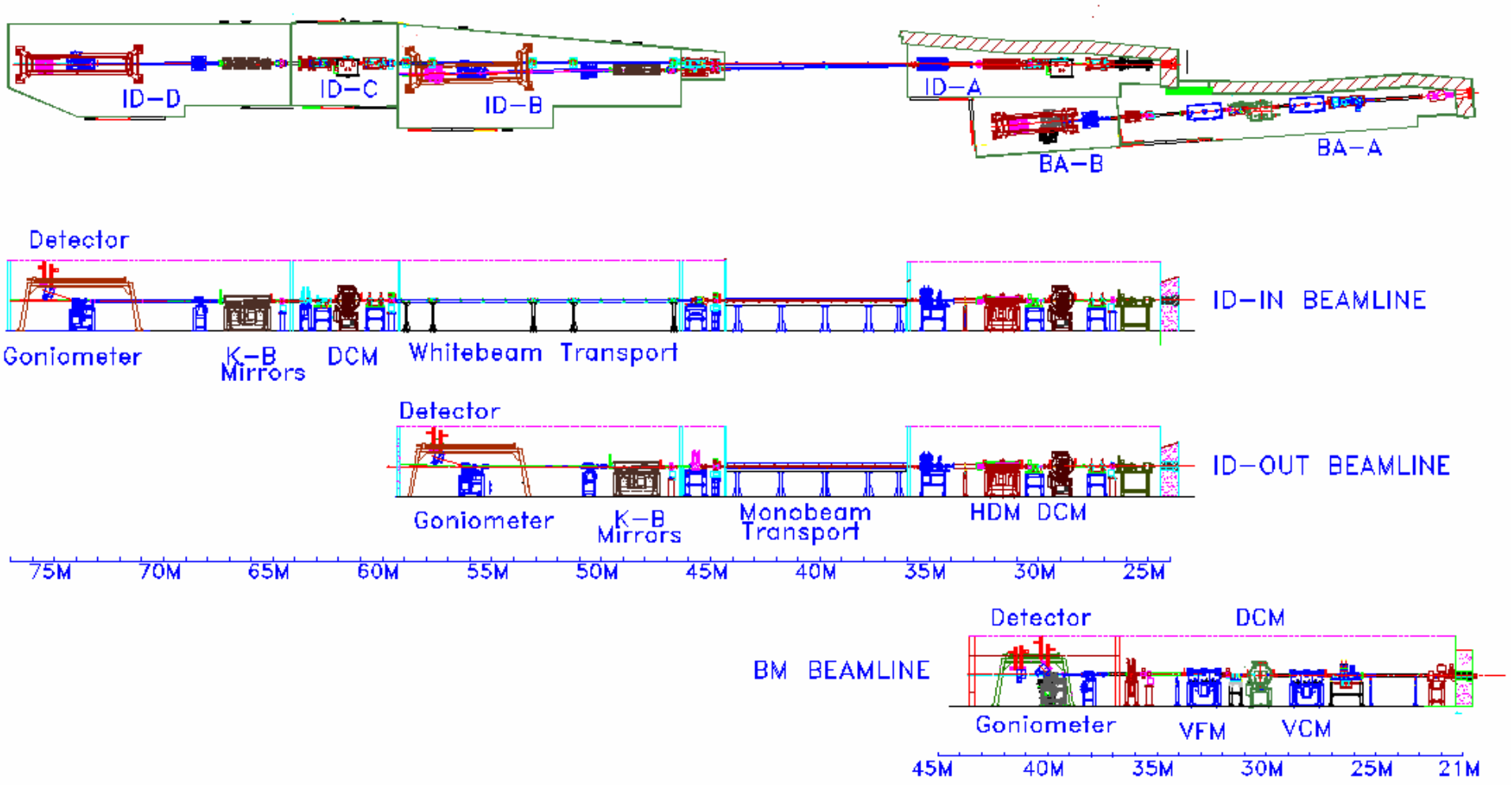
- Lots more screening – small crystals in particular
- Remote access
- Opens the door for project stuck on the shelf because of small or weakly scattering crystals

Structural Biology Beamlines at the Advanced Photon Source



Percent of time available to general users

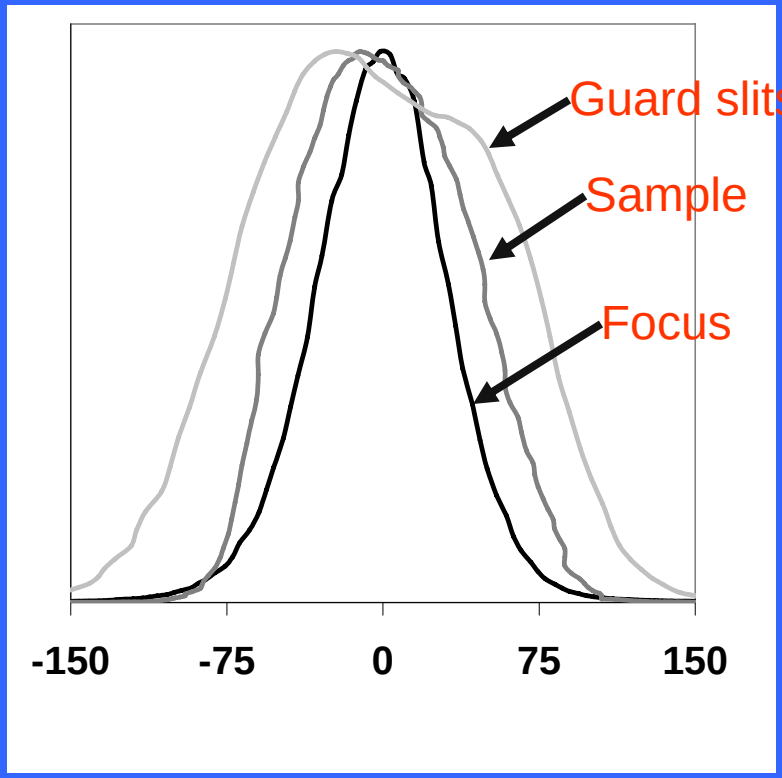
GM/CA Sector Layout



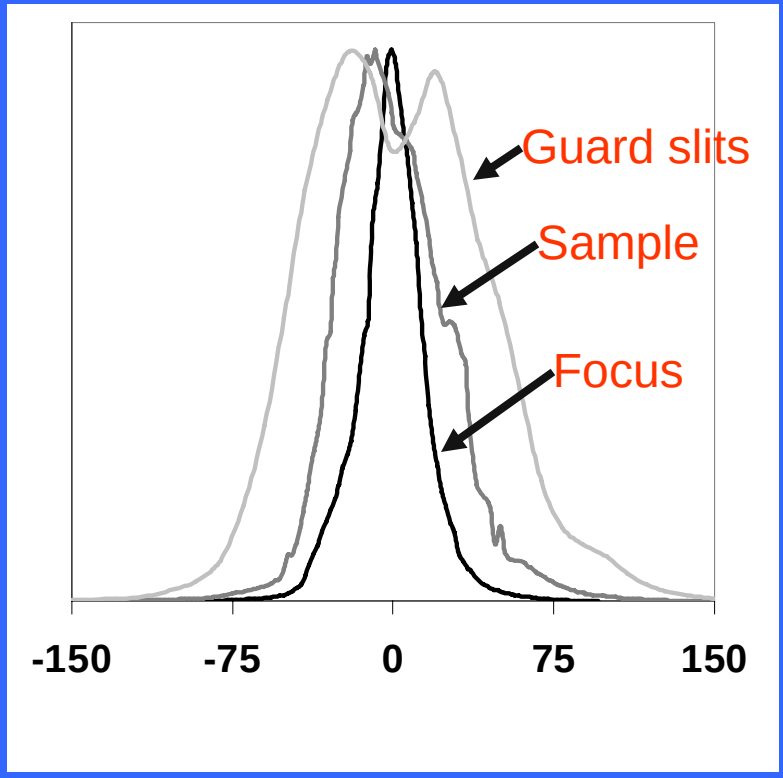
K-B "Bimorph" Mirrors Performance - off focus beam profiles

Focus at 72.3 m : 0 mm
Sample at 72.0 m : 300 mm
Guard slits at 71.8 m : 500 mm

Horizontal beam shape

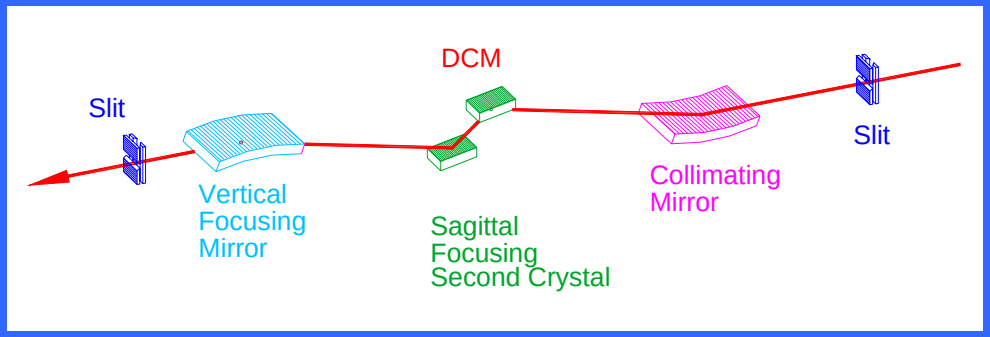


Vertical beam shape

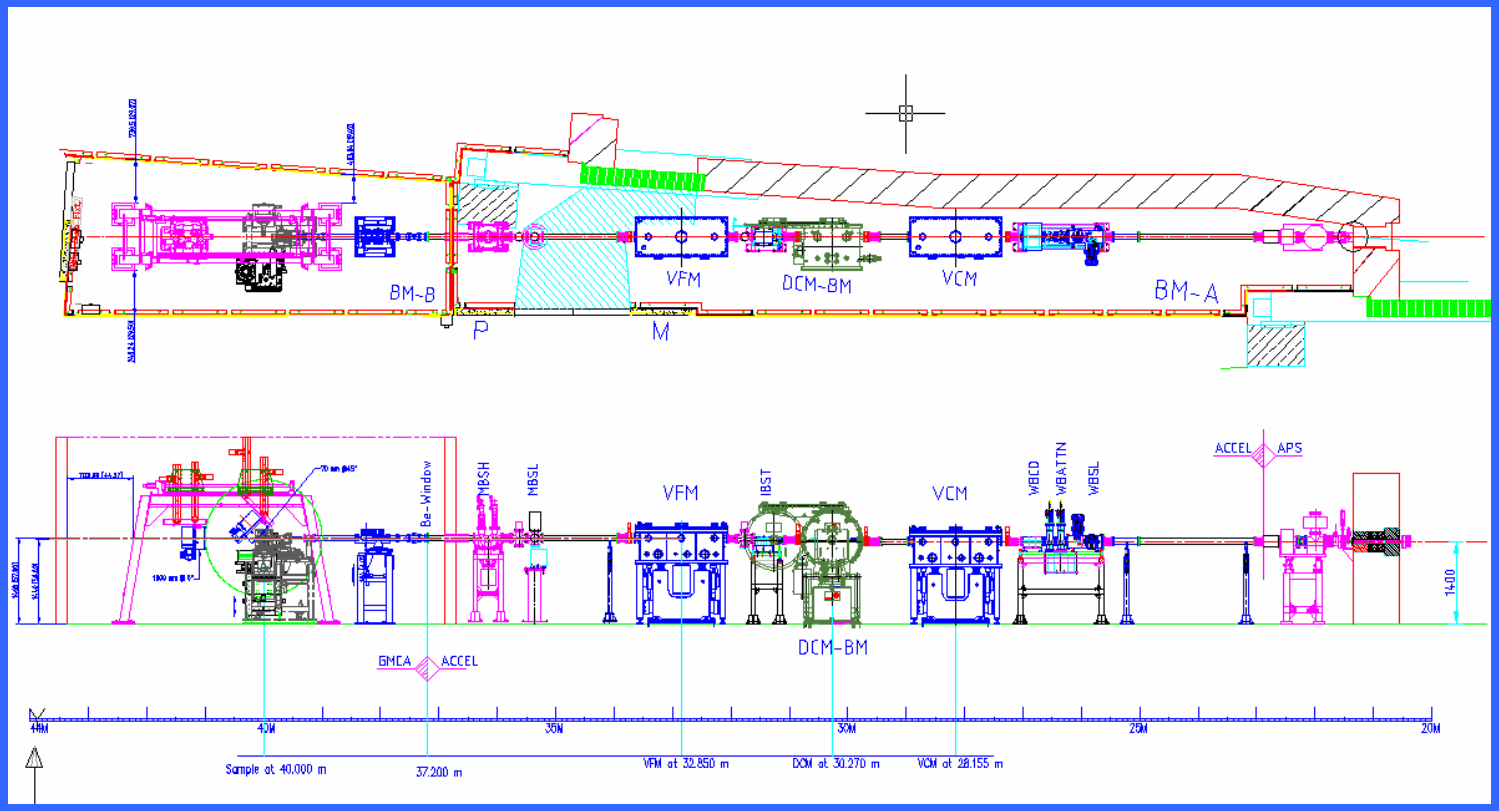


Only half of the electrodes are independent
More power supplies installed January 2007

Schematic of BM Beamline

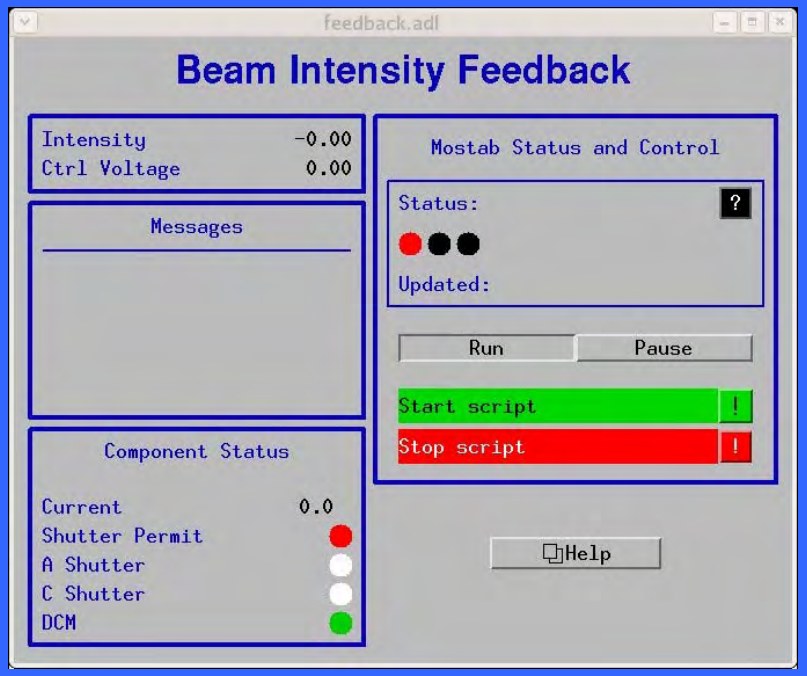


Energy Range: 3.5 – 25 keV
Flux: 0.5 – 1.2 x 10^{12} photons/sec/100mA
Focal size: 110 x 150 microns
Status: commissioning



Beamline Automation

- Parallel homing: brings beamline to life in less than 10 minutes
- FPE audit records actual exposure time and dose per frame
- Beam intensity feedback stabilizes I(0)
- Beam positional feedback locks position
- Fast-scan scripting library written for automated alignment & fast XAS scans
- Auto-alignment of optics



Gallery of PDB deposits from IDin

70 deposits for which all or part of data were collected at 23IDD or 23IDB lines